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# Degradation, Metabolism and Toxicity of Synthetic Pyrethroids

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**Synthetic pyrethroidal compounds undergo biodegradation in mammals both oxidatively and hydrolytically, and depending on the type of compound, either of the pathways may predominate. Thus, (+) - or (±) -*trans* isomers of the chrysanthemumate ester of primary alcohols such as fenothrin, furamethrin, proparthrin, resmethrin, and tetramethrin (and possibly permethrin, too) are metabolized mainly through hydrolysis of the ester linkage, with subsequent oxidation and/or conjugation of the component alcohol and acid moieties. On the other hand, the corresponding (+)-*cis* enantiometers and chrysanthemumate of secondary alcohols like allethrin are resistant to hydrolytic attack, and biodegraded via oxidation at various sites of the molecule. These rapid metabolic degradations, together with the presumable incomplete absorption from the gastrointestinal tract, would generally contribute to the low acute toxicity of synthetic pyrethroids.**

**These compounds are neither skin irritants nor skin sensitizers, and inhalation toxicity as well as dermal toxicity are fairly low. Neither is teratogenic in rats, mice, and/or rabbits or mutagenic on various bacterial strains. Subacute and chronic feeding of higher amounts of the compounds to rats invariably causes some histopathological changes in liver; however, these are neither indicative nor suggestive of tumorigenicity.**

**Based on existing toxicological information, the present recommended use patterns might afford sufficient safety margin on human population.**

**However, in extending usage to agricultural pest control, much more extensive investigations should be forthcoming from both chemical and biological aspects, since there is scant information on the fate of these pyrethroids in the environment. Also several of the compounds may be very toxic to certain kinds of fish and arthropods.**

## Introduction

Long-lasting, extensive efforts in many laboratories in modifying chemical structure of natural pyrethrins to obtain derivatives with better chemical properties and biological performances, have proved to be gradually successful, and numerous new compounds have been elaborated *(1—12)*. Some compounds are quite different from natural pyrethrins in their alcoholic moiety, and others even lack the cyclopropane ring in the acid moiety. Among these synthetic pyrethroidal compounds, allethrin and tetramethrin (phthalthrin) have been used commercially for more than 10 years; and recently, resmethrin and furamethrin have come to be used mainly for the control of insects of medical importance. The present worldwide tendency is, it seems, to develop pyrethroidal compounds for agricultural purposes, and fortunately, there exist already some promising candidates, such as fenothrin (phenothrin), permethrin and S5602.

However, before these pyrethroids are actually used for agricultural pest control, there are many problems to be solved. Aside from economics, that is, cost performance considerations to compete with organophosphorus compounds and carbamate insecticides, possible adverse effects of those compounds on humans as well as on the environment have not yet been fully assessed. One reason might be that, at present, regulational requirements for insecticides for household usage are much less stringent in most countries, as compared with requirements for petition of agricultural pesticides.

So, in the present discussions the existing information on the metabolism and toxicology of the

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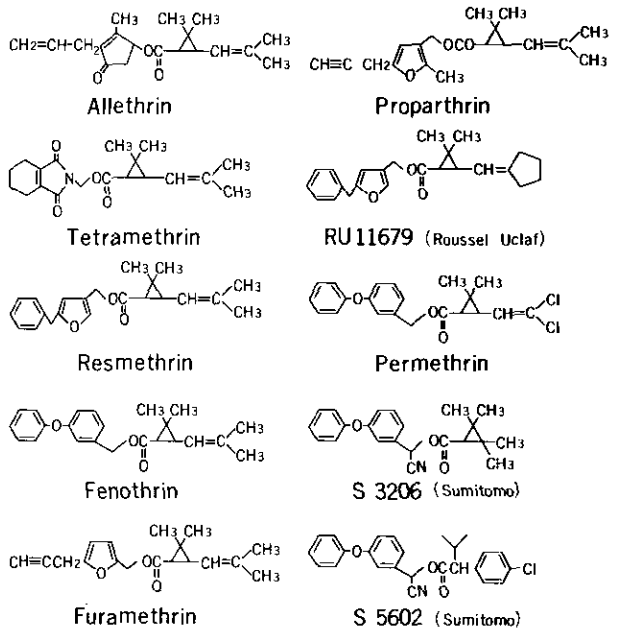



synthetic pyrethroids in nontarget species should be summarized as the basis for further investigations to be carried out to minimize the possible adverse effects of the compounds in the use of both public health purposes and agriculture. Natural pyrethrins will be dealt with only briefly, because they were already discussed in detail in the Symposium on Pyrethrum held in August 1972 *(13)*.

## Metabolism in Mammals

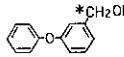
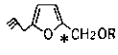
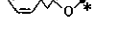
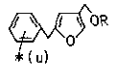
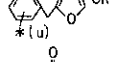
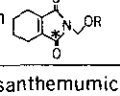
The historical stages in attempts at elucidating the metabolism of pyrethroids were critically reviewed by O'Brien *(14)* and also by Yamamoto *(15)*. Regrettably, none of these studies are of much significance now because of the unavoidable technological limitations in those days.

These metabolic studies became very much modernized in late 1960's, when the use of the radioactive compounds labeled with carbon-14 or tritium at known positions, coupled with refined separation techniques as well as application of the most sophisticated instrumentation like NMR and GLC-mass spectrometry, made possible separation and identification of a minute amount of metabolites. Knowledge of various enzyme systems capable of metabolizing foreign compounds in animals has contributed much to the understanding of the mechanism of biodegradation of pyrethroids. As a consequence, a fairly large amount of information has been acquired which can be summarized as follows.

When a radioactive pyrethroid is administered orally to mammals, it is absorbed from the intestinal tract of the animals and distributed in every tissue examined. The rate of absorption seems to be fairly rapid, because both whole body autoradiography and radioactivity determination in animal tissues revealed that the maximum concentration of radioactivity in the animal body is attained about 3 hr after administration, and thereafter the radioactivity disappears gradually from the tissues *(16—20;* Miyamoto and Kohara; unpublished observation 1972). The presumption is supported by the rapid appearance of radioactivity in the bile of rats orally treated with tritium-labeled proparthrin *(19)*. Major portions of the tissue radioactivity were accounted for by degradation products other than the intact pyrethroid, although the ratio was variable with the compound tested.

The excretion pattern of the radioactivity is summarized in Table 1 *(16—20;* Miyamoto and Kohara; unpublished observation, 1972; Miyamoto and Suzuki; unpublished observation, 1975). The rate of excretion was different depending upon the compound and also on the dosage. Roughly speaking, the radioactivity is excreted somewhat more slowly than with organophosphates and carbamates. The cumulative excretion pattern of *cis* and *trans* isomers was different from each other in

**Table 1. Excretion of radioactivity by oral administration of pyrethroids to rats.**

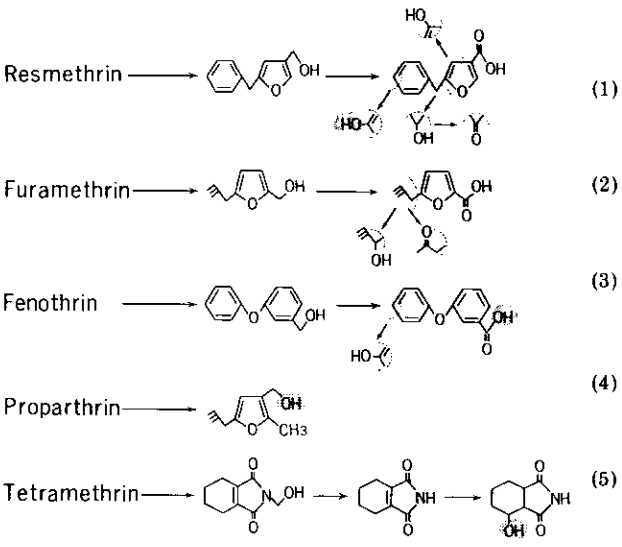
| Compound | Label (*14 c) | Dosage, mg/kg | Interval, days | Excretion % urine | feces | total |
|---|---|---|---|---|---|---|
| Fenothrin (+*trans*) |  | 200 | 3 | 57 | 44 | 101 |
| Fenothrin (+*cis*) |  | 200 | 3 | 15 | 65 | 80 |
| Furamethrin (±*trans*) |  | 1000 | 7 | 47 | 47 | 94 |
| Resmethrin (±*trans*) |  | 500<br>500 | 7<br>20 | 30<br>36 | 51<br>64 | 81<br>100 |
| Resmethrin (+*trans*) |  | 1.1 | 6 | 38 | 15 | 53 |
| Resmethrin (+*cis*) |  | 1.1 | 6 | 17 | 52 | 69 |
| Tetramethrin (±*trans*) |  | 500 | 5 | 50 | 45 | 95 |

R; chrysanthemumic acid moiety

resmethrin and fenothrin. In some compounds the radioactivity was not completely recovered in the excreta. Although not reproduced here, 40% and 35% of tritium was eliminated in 4 days in urine and feces, respectively, of rats given oral doses of 100 mg/kg of furylmethyl-$^3$H proparthrin (*19*). An appreciable amount of tritium from allethronyl-$^3$H (+)-*trans*-chrysanthemumate was not recovered in the rat excreta (*21,22*). Incomplete excretion was also observed in the acid moiety of resmethrin, 70—73% of the radiocarbons being recovered during 6-day intervals (*20*).

A substantial amount of the radioactivity was found in feces in every compound tested regardless of the dosage, and enterohepatic circulation may play some role, as in the case of resmethrin *(17)* and proparthrin *(19)* . Massive administration of some phyrethroids resulted in elimination of unmetabolized parent compound. for example, 9% and 15% of the intact *trans*- and *cis*-fenothrin, respectively, and 16% of tetramethrin relative to the amount applied were found in feces at the dosage in Table 1. No unmetabolized furamethrin or resmethrin was excreted regardless of the dosage, possibly due, in part, to the intrinsic instability of the compound. So, some portions of the pyrethroid might be excreted unabsorbed, and this might be one reason for low toxicity of pyrethroid in mammals. Anyway, more intensive examination should be desirable to ascertain whether or not the degradation products remain in the body of the animal.

Many of the above *trans* isomers have been found to be metabolized mainly through cleavage at the ester linkage to give the corresponding alcohols which were subsequently oxdized to the carboxylic acids, as shown in eqs. (1)—(5) (grey circle indicates site of conjugation). Under these *in vivo* conditions, however, no such component alcohol from resmethrin, furamethrin, or fenothrin was ever detected in the urine except glucuronide conjugate of proparthrin alcohol, although 5-benzyl-3-furylmethanol or 3-phenoxybenzyl alcohol was present in the animal tissues and blood *(17—20;* Miyamoto and Kohara; unpublished observation, 1972). These carboxylic acids were further hydroxylated and/or conjugated, and excreted: in the case of resmethrin, approximately two-thirds of the urinary radioactivity was accounted for by these 5-carboxylic acids (free and conjugated) *(17)*, and one-half of the urinary radiocarbons derived from furamethrin and fenothrin comprised 5-(2-propynyl) 2-furoic acid plus its 2-oxidation products and 3-phenoxybenzoic acid plus 3-(4-hydroxy phenoxy)benzoic acid, respectively (*18*: Miyamoto and Kohara; unpublished observation, 1972). (±) -

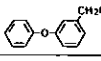
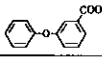



*Trans*-tetramethrin gave as a major metabolite 3-hydroxycyclohexane-1,2-dicarboximide produced via 3,4,5,6-tetrahydrophthalimide, which in turn had been derived from *N*-(hydroxymethyl)-3,4,5,6-tetrahydrophthalimide (*16, 23*).

Fecal metabolites of these *trans* isomers as well as the remaining urinary radiocarbons have not been well characterized, due in part to the intrinsic instability of the compounds and also due to numerous numbers of minor metabolites.

The cleavage reaction of the ester linkage is catalyzed by esterases which are mainly localized in liver microsomal fractions of various mammalian species *(23,24)*. Table 2 shows that one or more esterases are present in all the mammalian species tested *(18)*. Not only fenothrin shown here,

**Table 2. Metabolism of (+)-*trans*-fenothrin by 8000 g supernatant of liver homogenate from several species of mammals.[a]**

| Animal species | Metabolite formed (%) | | | |
|---|---|---|---|---|
| | Fenothrin metabolized |  |  | Other ether-solubles |
| Dog | 33.9 | 24.9 | 3.5 | 5.5 |
| Guinea pig | 39.2 | 26.8 | 1.3 | 8.1 |
| Mouse | 21.1 | 10.9 | 1.2 | 8.9 |
| Rabbit | 31.7 | 16.3 | 8.1 | 7.3 |
| Rat | 24.9 | 13.9 | 2.5 | 8.5 |

[a]A mixture containing 250 μmole of potassium phosphate buffer (pH 7.5), 5 μmole of fenothrin, and the 8000 g supernatant equivalent to 0.35 g liver in 5 ml was incubated for 60 min at 37.5°C. Sum of the recovered radioactivity (including water solubles and precipitate) is referred to as 100%.

but all (+)- and (-)-*trans* isomers of resmethrin, tetramethrin, and permethrin are well hydrolyzed by the enzymes *(25—27)*. Organophosphorus compounds such as *S,S,S*-tributyl phosphorothiolate, paraoxon, malaoxon and NIA16388 as well as insecticidal carbamates inhibit the hydrolysis reaction *(23,25,26,28)*. Malathion also inhibits hydrolysis of fenothrin and vice versa, and the identity of pyrethroid esterases with malathion carboxylesterase is suspected *(27)*.

What is interesting here is that none of the *cis* isomers tested, whether it be the (+) - or (-)-enantiomer, is a good substrate for the esterases, and they are hydrolyzed at a far slower rate, as shown in Figure 1 *(18, 24–26)* (also Table 3). As is evident from the results, (+)-*cis*-resmethrin is hydrolyzed at a rate one-tenth of that of (+)-*trans* resmethrin by mouse hepatic esterases. More or less similar relationships hold true for the isomers of fenothrin, permethrin, and tetramethrin. *In vitro*, the *cis*

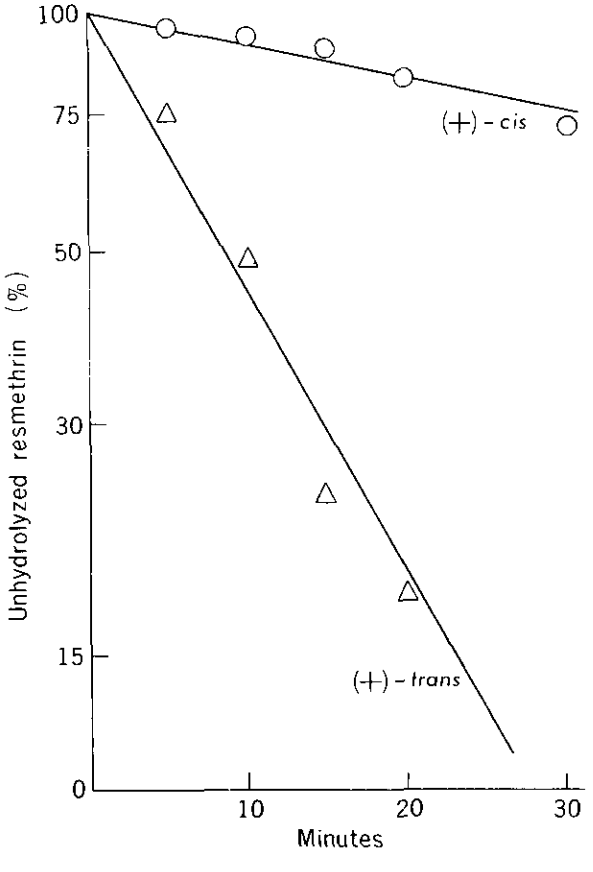



FIGURE 1. Specificity of esterases for hydrolysis of (+)-*trans*-resmethrin and (+)-*cis*-resmethrin. Acetone powder preparation of mouse liver microsomes (17.8 mg) and the pyrethroid (100 nmole) in 2.5 ml of Tris-HCl buffer (50*mM*, pH 7.5). Incubation temperature, 37°C.

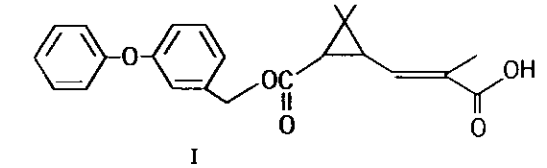
**Table 3. Hydrolysis of 3-phenoxybenzyl- and α-cyano-3-phenoxybenzylchrysanthemumates and their dihalogenated acid derivatives by rat liver microsomal fractions.[a]**

| Compound | | Relative hydrolysis rate, % | |
|---|---|---|---|
| acid ↓ | alcohol → | HO-⟨⟩-O-⟨⟩ | CN HO-⟨⟩-O-⟨⟩ |
| (+) -trans- | >=-⟨⟩-CO2H | 100 | 3.0 |
| (+) -cis- | 〃 | 1.7 | 0.2 |
| (+) -trans- | Cl, Cl >=-⟨⟩-CO2H | 135 | 10.7 |
| (+) -cis- | 〃 | 1.8 | 0.0 |
| (+) -trans- | Br, Br >=-⟨⟩-CO2H | 66 | 3.1 |
| (+) -cis- | 〃 | 1.1 | 0.0 |

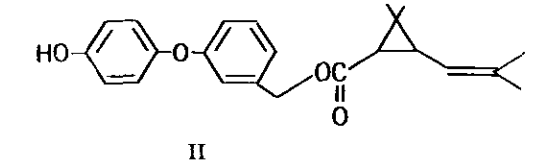
[a]A 2.5 μmole portion of each pyrethroid was incubated in 5 ml with a 20 mg protein equivalent rat liver microsomal fractions at pH 7.5 for 30 min (*trans*) or 60 min (*cis*) at 37.5°C. Hydrolysis rate of (+)-*trans*-fenothrin (91 μmole/hr-mg protein) is referred to as 100%.

isomers of these pyrethroids are degraded oxidatively by NADPH-fortified mfo *(23,26)*.

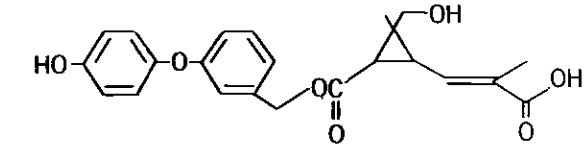
In accord with the *in vitro* studies, *in vivo* experiments revealed that (+)-*cis*-resmethrin gave rise to at least two unidentified ester metabolites in rat feces *(20)*. Feces of rats contained three ester metabolites (I—III) of (+) - *cis*-fenothrin which are never or hardly derived from (+) - *trans*-fenothrin. These three metabolites have the ester linkage intact; and I is oxidized at the methyl group in the isobutenyl moiety of chrysanthemumic acid, II is hydroxylated in the benzene ring of the phenoxybenzyl alcohol moiety, and III has been



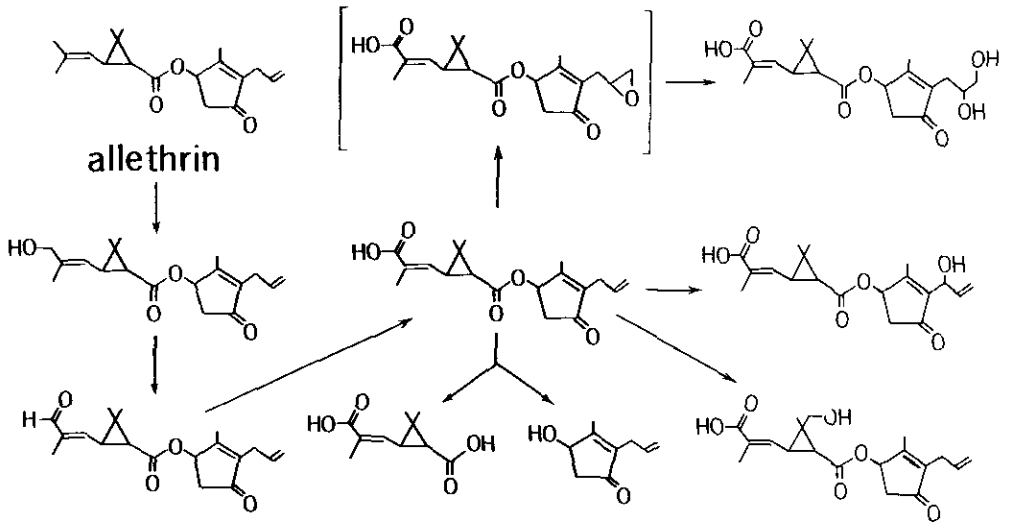

I




II




III




(6)

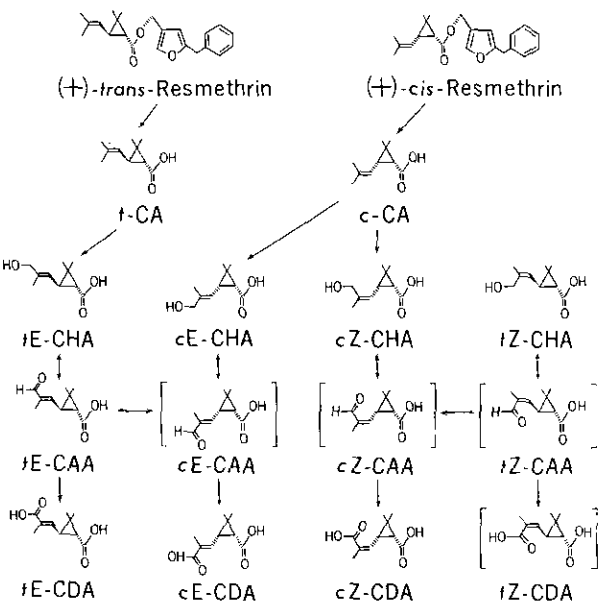
oxidized at three positions in the fenothrin molecule (Miyamoto and Suzuki; unpublished observation, 1975).

However, some of the *trans*-chrysanthemumate esters are not hydrolyzed either by mammalian esterases. For example, once the cyano group is introduced to the $\alpha$-carbon of phenoxybenzyl alcohol of *trans*-fenothrin, the resultant ester is hardly hydrolyzed, as shown in Table 3 *(27)*.

Similarly, pyrethrin-I or allethrin, which is the *trans* ester of the secondary alcohol, is very resistant to hydrolytic attack *in vitro* as well as *in vivo* *(21,22,25)*. As a result, when radiolabled allethrin was orally administered to rats, several ester metabolites were obtained in rat urine, as shown in the reaction scheme (6). Although chrysanthemumic acid and allethrolone were detected as minor metabolites, the major ones are those oxidized at methyl groups in the acid moiety or at allyl group in allethrolone alcohol.

As to the *in vivo* metabolism of acid moiety of the synthetic pyrethroids, few studies have so far been carried out *(20)*. Interesting findings have been obtained, by Ueda et al. *(20)*, as shown in eqs. (7). Namely, one-third of acid-derived metabolites of (+)-*trans*-resmethrin administered to rats were characterized and among them *cis*-hydroxymethyl chrysanthemumic acid (cE-CHA) and *cis*-chrysanthemum dicarboxylic acid (cE-CDA) were identified, amounting to approximately 10% of the administered radioactivity. On the other hand, (+)-*cis*-resmethrin yielded *trans* isomers of the two acids (tZ-CHA, tZ-CDA), but the epimerization was much less extensive. The aldehyde (CAA) is presumed to be the most likely intermediate for isomerization in the metabolic sequences, although the presence of the aldehyde was not positively demonstrated.

These are brief summaries of *in vivo* metabolism of the synthetic pyrethroids in mammals. Most of the key reaction sequences are confirmed by *in vitro* enzymatic studies, although they are not dealt with in more detail. Thus, pyrethroid compounds are biodegraded both oxidatively and hydrolytically, depending on the type of compound; on the basis of the results mentioned above, all these pyrethroids can be presumed to be extensively degraded in mammals.




(7)

## Fate in the Environment

Environmental studies such as photodecomposition, decomposition on and in soil, as well as plant metabolism are prerequisites for agricultural application of the pyrethroids. However, few investigations have been undertaken hitherto, and especially none on plant and soil metabolism.

Photodecomposition studies were carried out with [carboxy-$^{14}$C] acid-labeled (+)-*trans*-allethrin, -tetramethrin, -dimethrin, and pyrethrin-I in a thin film on glass and irradiated with a sunlamp at 40°C in air *(29)*. The rate of decrease of these pyrethroids varied considerably with alcohol moiety, the time required for 90% decrease being 0.2, 4, 8, and 16 hr for pyrethrin-I, tetramethrin, allethrin, and dimethrin, respectively. The ester linkage was intact and saponification of the photoproducts yielded more than ten acids, among which six were identified, including *trans*-chrysanthemumic acid. The results indicate that the photochemical changes in the acid moiety common to 4 pyrethroids involve at least stepwise oxidation of the *trans*-methyl group of the isobutenyl moiety to the respective alcohol, aldehyde, and carboxylic acid derivatives; oxidation of the isobutenyl double bond to a keto derivative, and rupture of this double bond to yield the ester of *trans*-caronic acid. In another study *(30)*, (+)-*trans*-tetramethrin gave on shorter irradiation primarily *R* and *S* isomers of *trans*-epoxytetramethrin. (+)-*Trans*-resmethrin is much more labile than (+)-*trans*-tetramethrin or (+)-allethronyl (+)-trans-chrysanthemumate *(30,31)*. Not only is epoxidation of the isobutenyl double bond apparent, but also photolytic reactions are observed, such as cleavage of the ester bond and oxidation of the furan ring yielding a cyclic ozonide-type peroxide intermediate, with subsequent formation of several ester derivatives. The (+)-*trans*-chrysanthemumic acid, benzoic acid, phenylacetic acid, and benzyl alcohol are detected, although 5-benzyl-3-furylmethanol, the component alcohol, is not present. (+)-*Cis*-resmethrin decomposed at similar rate. Photoisomerization of chrysanthemumic acid never occurred under any conditions *(30)*. Thus, aside from various photoalteration reactions in the alcohol moiety of each pyrethroid, the chrysanthemumic acid moiety has two photolabile groups, the isobutenyl double bond and the terminal methyl group, which render chrysanthemumate esters short-lived in the environment.

Permethrin, which is devoid of the photolabile terminal methyl groups in isobutenyl moiety, is much more stable than other pyrethroidal compounds *(32,33)*. Under indoor conditions (facing south), where (+)-*trans*-resmethrin was decomposed within a few hours, a halflife of permethrin in thin film was more than 20 days. Fenothrin was intermediate, with a halflife about 6 days. When exposed to outdoor sunlight, a significant residue of permethrin remained undecomposed after 10 days, while (+)-*trans*-resmethrin decomposed very rapidly (within 24 hr) and fenothrin in 10 days.

## Toxicity in Mammals

Although some of the synthetic pyrethroids as well as the natural pyrethrin mixture have a long history of usage without any apparent hazards to humans, only a few toxicity data on these synthetic pyrethroids are available in the literature, so far as is known. Therefore, those data that have been obtained mostly in our laboratory by using our technical materials are summarized here (Miyamoto, Kadota, and Kohda; unpublished observations 1969—1975; Miyamoto and Suzuki; unpublished observation, 1975; Miyamoto, Takimoto, and Kagoshima; unpublished observations, 1975). They include from acute toxicity data to chronic feeding and mutagenicity screening results. The chemical purity of the preparation used ranged from 93 to 100%, and the optical purity was more than 98%. The *trans, cis* ratio of the racemic mixture was 4:1 in all the compounds except permethrin, where the ratio was roughly 3:2.

### Acute Oral Toxicity

In acute oral toxicity studies the test compound was dissolved in corn oil, and 0.1 ml/10 g body weight and 0.5—1 ml/100 g body weight were administered by stomach tube to dd strain mice and Sprague Dawley rats, respectively. Some liquid compounds were administered without a vehicle. The toxic symptoms were hypersensitivity, tremors, and then motor ataxia in both mice and rats; also bloody tears and urinary incontinence were noted in rats. The onset of the symptoms was observed 30—60 min after treatment in mice, and in rats 2—3 hr after administration. The surviving animals recovered in 24 hr in the case of mice and in 2—3 days in the case of rats. The onset of the symptoms as well as their disappearance were delayed when permethrin was administered. The animals were kept for 1 week and $LD_{50}$ values were calculated according to Litchfield and Wilcoxon *(34)*. Table 4 shows the $LD_{50}$ of each compound. The figures in parentheses indicate the mortality at the highest dose. Mice are invariably more susceptible than

rats, and female rats are more susceptible to allethrin than males. When the toxicity in mice of (+)-*trans* isomers of the synthetic pyrethroids is compared with natural pyrethrin mixture, they are less toxic than pyrethrin. Fenothrin is least toxic, followed by permethrin and furamethrin. Among the (+)-*cis* isomers tested, (+)-*cis*-permethrin, resmethrin and -allethrin are more toxic than their *trans* counterparts, which is consistent with the previous data (*35*). Easier hydrolyzability of (+)-*trans* isomers mentioned earlier might contribute to the lower toxicity to mammals as compared with (+)-*cis* isomers. Pretreatment of the animals with organophosphorus compounds actually enhances the toxicity of (+)-*trans* isomers (*25;* Miyamoto and Suzuki; unpublished observation, 1974). However, the extent to which the hydrolytic cleavage is related to the toxicity of these compounds has not been assessed. (−)-Isomers are generally less toxic than the corresponding (+)-enantiomers. The oral toxicity changes to a certain degree depending on the vehicle solvent (*25*; Miyamoto and Kadota; unpublished observation, 1974). In fact, some pyrethroidal compounds are very toxic to mammals on intravenous administration (*15, 16, 34*). As pointed out earlier, the rate of absorption might be one of the major factors controlling the low toxicity of the pyrethroids.

**Table 4. Acute oral toxicity of synthetic pyrethroids.**

| Compound | $LD_{50}$, mg/kg[a] | | | |
|---|---|---|---|---|
| | Mice | | Rats | |
| | Male | Female | Male | Female |
| Pyrethrin[b] | 370 | 500 | 2160 | 1300 |
| Allethrin | | | | |
| Racemic | 500 | 630 | 2430 | 720 |
| (+)-*trans* | 330 | 350 | | |
| (+)-*cis* | 210 | 270 | | |
| (+)-allethronyl (+)-*trans* | 285 | 250 | 1290 | 430 |
| Fenothrin | | | | |
| Racemic | >5000(0) | >5000(0) | >10000(0)[c] | >10000(0)[c] |
| (+)-*trans* | >5000(0) | >5000(0) | | |
| (+)-*cis* | >2500(0) | >2500(0) | | |
| (−)-*trans* | >5000(0) | >5000(0) | | |
| (−)-*cis* | >5000(0) | >5000(0) | | |
| Furamethrin | | | | |
| Racemic | 2650 | 2100 | >10000(0)[c] | >10000(0)[c] |
| (+)-*trans* | 1700 | 1700 | | |
| Permethrin | | | | |
| Racemic | 490 | 490 | > 5000(20) | > 5000(0) |
| (+)-*trans* | 3100 | 3200 | | |
| (+)-*cis* | 107 | 85 | | |
| (−)-*trans* | >5000(0) | >5000(0) | | |
| (−)-*cis* | >5000(0) | >5000(0) | | |
| Resmethrin | | | | |
| Racemic | 690 | 940 | >5000(0) | >5000(0) |
| (+)-*trans* | 590 | 800 | | |
| (−)-*cis* | 152 | 160 | | |
| (−)-*trans* | 500 | 600 | | |
| (−)-*cis* | 3700 | 5000 | | |
| Tetramethrin | | | | |
| Racemic | 1920 | 2000 | > 5000(0) | > 5000(0) |
| (+)-*trans* | 930 | 910 | | |
| (+)-*cis* | >1000(0) | >1000(0) | | |

[a]Figures in parentheses indicate the % mortality at the highest dosage.
[b]As 20% extract, administered as such, and calculated in terms of active ingredient.
[c]Administered without vehicle.

## Dermal Toxicity

Dermal toxicity of the racemic form and/or (+)-*trans*, (+)-*cis* mixture of these pyrethroids was tested in mice and rats. By the highest possible dosage of each compound that can be applied experimentally, mortality was less than 50%, and even $LD_{50}$ of one of the most toxic compounds, (+)-allethronyl (+)-*trans*-chrysanthemumate, is above 2500 mg/kg in mice. These results indicate that dermally the pyrethroids are even less toxic than by oral administration.

None of the synthetic pyrethroids are irritants to either rabbit skin or to rabbit eyes according to the proposed criteria (36), with only a slight, transient congestion of conjunctiva or lacrimation by massive instillation of such compounds as allethrin, fenothrin, and tetramethrin.

Neither of the synthetic pyrethroids is a skin sensitizer in guinea pigs, when tested by the prescribed method [intracutaneous administration every other day for 10 times and 14 days thereafter intracutaneous injection for challenging *(37)*]. By patch test with 200 human volunteers, tetramethrin proved to be neither a primary irritant nor a sensitizer to the human skin (Weir; private communication, 1966).

## Inhalation Toxicity

Inhalation toxicity is very important in the study of pyrethroids, because these compounds are mainly used as the active ingredients of aerosols and mosquito coils for household insect control. Although subacute inhalation toxicity data of all such formulations are available, only toxicity data of mist preparation are presented here.

Fine particles of each pyrethroid with a diameter of 1—2 $\mu$m generated by means of an atomizer were introduced into an exposure chamber. Thus, rats and mice were exposed continuously to a mist of pyrethroid at different aerial concentration in the dynamic way for the specified intervals. Even at the highest aerial concentrations (Table 5), no death of the animals was recorded, except with (+)-allethronyl (+)-*trans*-chrysanthemumate, although toxic symptoms like hypersensitivity, motor ataxia and urinary incontinence were observed during heavy exposure of each compound. Some compounds were not tested at higher concentrations than that in Table 5 due to the physical properties of the solution. When the toxicity of each compound was compared in term of minimal toxic concentration, namely, the lowest concentration causing the symptoms of intoxication, allethrin and permethrin are slightly more toxic than other compounds.

Subacute inhalation toxicity testings conducted, as shown in Table 6, under similar experimental conditions for 3—4 hr/day for up to 4 weeks revealed no significant, compound-related findings in the examinations listed, including hematology, clinical biochemistry, and microscopic histopathology of major organs and tissues. The

**Table 5. Acute inhalation toxicity of synthetic pyrethroids.**

| Compound | Experimental conditions | | | $LC_{50}$, mg/m$^3$ | | Minimum toxic dose, mg/m$^3$ | |
|---|---|---|---|---|---|---|---|
| | Solvent | Exposure, hr | Air flow, l./min | Rats (M,F)[a] | Mice (M,F)[a] | Rats | Mice |
| Allethrin | | | | | | | |
| Racemic | Kerosine | 2 | 35 | >2000 | >2000 | 260 | 260 |
| (+)-*trans, cis* | Kerosine | 2 | 35 | >2000 | >2000 | 260 | 260 |
| (+)-allethronyl (+)-*trans* | Kerosine | 3 | 50 | 1600 | 2720 | 24 | 91 |
| Fenothrin | | | | | | | |
| (+)-*trans,cis* | Kerosine | 4 | 50 | >1200 | >1200 | 1200 | 1200 |
| Furamethrin | | | | | | | |
| (+)-*trans,cis* | Kerosine | 2 | 50 | >2000 | >2000 | 450 | 450 |
| Permethrin | | | | | | | |
| racemic | Kerosine | 3 | 50 | > 685 | > 685 | 140 | 140 |
| Resmethrin | | | | | | | |
| (+)-*trans,cis* | Kerosine/ xylene(9/1) | 4 | 50 | >1500 | >1500 | 400 | 400 |
| Tetramethrin | | | | | | | |
| Racemic | Methyl-chloroform | 3 | 35 | >2500 | >2500 | 2000 | 2000 |

[a]Males and females.

maximum no-effect concentration of each pyrethroid is much higher, generally more than 100-fold, than the aerial concentration attained in practical applications.

## Teratology

Teratology studies on several synthetic pyrethroids were carried out in New Zealand white rabbits, ICR mice and Sprague-Dawley rats to examine maternal and embryotoxic effects such as abortion and resorption. Maximum dosage was determined by growth suppression and toxic symptoms in pregnant females. Usually 10 to 15 pregnant rabbits, 15 to 20 pregnant rats or mice per dose were used, and for breeding of offspring 7 extra pregnant animals were used. Pups were obtained by Caesarian section prior to the termination of gestation period, and external as well as skeletal abnormalities were examined. In some experiments indicated in Table 7, offspring were delivered naturally and they were kept for several weeks to check their growth and organ differentiation, like unfurling of the auricle, growth of the hair, incisor dentition, opening of eyelids, descent of the testes, opening of the vaginal orifice and development of Preyer and righting reflexes. No significant adverse effects were observed.

**Table 6. Subacute inhalation toxicity of synthetic pyrethroids.[a]**

| Compound | Dose mg/m³ | Exposure hr/day | days/week | weeks | Findings[a] |
|---|---|---|---|---|---|
| Allethrin | | | | | |
| (+)-*trans,cis* | 123 | 3 | 5 | 4 | Toxic symptoms |
| (+)-allethronyl (+)-*trans* | 6.1, 16.9, 61.3 | 3 | 5 | 4 | Toxic symptoms (61.3 mg/m³) |
| Fenothrin, (+)-*trans, cis* | 41, 63, 210 | 4 | 5 | 4 | |
| Furamethrin, (+)-*trans, cis* | 20, 40, 200 | 3 | 5 | 4 | Toxic symptoms (200 mg/m³) |
| Resmethrin, (+)-*trans,cis* | 23, 47, 210 | 4 | 5 | 4 | |
| Tetramethrin, racemic | 200 | 3 | 6 | 4 | |

[a]Mice and rats (both male and female) were exposed.
[b]The following items were examined: mortality, behavior, food intake; hematology (erythrocyte, hematocrit, hemoglobin, sedimentation rate, platelet, total and differential leucocyte); clinical biochemistry (Na, K, Ca, glucose, urea nitrogen, total protein, albumin, bilirubin, GOT, GPT, alkaline phosphatase); histopathology (brain, pituitary, thyroid, adrenals, liver, kidney, spleen, pancreas, testis/ovary, lung, trachea, eye, heart).

**Table 7. Teratology study of synthetic pyrethroids.**

| Compound | Animals | Dose, mg/kg-day | Route | Administration, days of gestation |
|---|---|---|---|---|
| Allethrin | | | | |
| Racemic[a] | Rabbits | 215, 35 | PO | 6–18 |
| (+)-*trans,cis* | Mice[b] | 15, 50, 150 | PO | 7–12 |
| (+)-allethronyl (+)-*trans* | Mice[b] | 10, 30, 100 | PO | 7–12 |
| Fenothrin, (+)-*trans, cis* | Mice[b] | 30, 300, 3000 | PO | 7–12 |
| | Rabbits | 10, 100, 1000 | PO | 6–18 |
| Furamethrin, racemic[d] | Mice | 10, 100 | IP | 7–12 |
| | Rats | 10, 100 | IP | 9–14 |
| Permethrin, racemic | Mice[b] | 15, 50, 100 | PO | 7–12 |
| Resmethrin, (+)-*trans,cis* | Mice[b] | 10, 30, 50 | PO | 7–12 |
| | Rats[b] | 10, 20, 50 | PO | 9–14 |
| Tetramethrin, racemic[e] | Rabbits[b] | 30, 90 | PO | 6–18 |

[a]Data of Weatherholtz (private communication, 1972).
[b]Includes breeding of naturally delivered offspring.
[c]Data of Yamamoto et al. (private communication, 1972).
[d]Data of Rutter (private communication, 1974).
[e]Data of Weir (private communication, 1966).

## Subacute and Chronic Toxicity

To assess subacute and chronic toxicity of the pyrethroids, groups of 12 to 20 each of males and females (in the case of chronic allethrin feeding each 30 males and females per group) were fed a diet containing the specified concentration of the compound. Table 8 summarizes the experimental designs and compound-related changes in several of these feeding testings. Examinations were made on behavior, mortality, growth, food intake and water consumption, and urinalysis during feeding, and at the terminal necropsy, on hematology, clinical biochemistry, major organ weight and microscopic histopathology of the following organs and tissues: brain, eye, spinal cord, peripheral nerve, bronchus, lung, heart, spleen, bone marrow, mesenteric lymph node, thymus, esophagus, stomach, small intestine, large intestine, salivary gland, liver, pancreas, kidney, urinary bladder, testis/ovary, prostrate/uterus, pituitary, thyroids, adrenals, skin, and any tissue mass.

At the higher concentrations these pyrethroids invariably cause a slight increase of liver weight, often accompanied by some histopathological changes such as hypertrophy, oval cell infiltration and bile duct proliferation. On taking into account similar histopathological changes reported (*38*) in liver of rats after 2 yr feeding of a natural pyrethrin mixture, liver damage is likely to be a common adverse effect of pyrethroidal compound. However, 80 week consecutive feeding of racemic allethrin to rats revealed upon very careful examinations no histopathological changes indicative or suggestive of hyperplasia in any tissue. The no-effect level after 6-month feeding ranges from 1000 to 2500 ppm of the compound in the diet. Although the data are not reproduced here, the no-effect level of tetramethrin in beagle dogs after dietary administration for 3 months was 5000 ppm (Weir; private communication, 1966).

## Mutagenicity Screening

As the final toxicological studies on the pyrethroids, the results of mutagenicity screening tests with several strains of bacteria are presented here. Two kinds of procedure were used for the test. In the first trial, tryptophanless or histidineless mutant cells were innoculated on the corresponding minimal agar plate, and at the center of the plate a small piece of filter paper containing the test compound was placed. If the compound is a

**Table 8. Subacute and chronic toxicity study of synthetic pyrethroids.**

| Compound | Animals | Dietary concentration, ppm | Duration, weeks | No-effect level, ppm | Findings (ppm)[b] |
|---|---|---|---|---|---|
| Allethrin | | | | | |
| Racemic | Rats, Wistar | 1000, 5000, 15000 | 12 | 1000 | (≥5000); body weight gain↓liver weight ratio↑ H (liver)[b] |
| Racemic[c] | Rats, Wistar | 500, 1000, 2000 | 80 | 500 | (≥1000); H (liver)[d] (2000): GOT↓ |
| (+)-allethronyl (+)-*trans* | Rats, SD | 500, 1500, 500 | 12 | 1500 | (5000); body weight gain↓ liver weight↑ |
| Fenothrin, (+)-*trans,cis* | Rats, SD | 1000, 2500, 5000, 10000 | 24 | 2500 | (≥5000); liver weight↑H (liver)[c] (10000); body weight gain↓ |
| Furamethrin, racemic | Rats, Wistar | 500, 100, 5000 | 24 | 1000 (M) | (≥1000 F), (5000,M) H (liver)[b] |
| | | | | 500 (F) | (5000); liver weight↑ |
| Permethrin, racemic | Rats, SD | 375, 750, 1500 | 24 | 1500 | (3000); toxic symptoms liver weight↑H(liver)[e] |
| Resmethrin (+)-*trans, cis* | Rats, SD | 500, 1500, 5000 | 24 | 1500 | (5000); toxic symptoms liver weight↑ALPase↑ |

[a] Ascending and descending arrows indicate increase and decrease, respectively.
[b] Bile duct proliferation and oval cell infiltration.
[c] Data of Ito et al. (private communication, 1973).
[d] Bile duct proliferation.
[e] Hypertrophy and fatty change.

mutagen, then colonies of the revertant appear on the plate. In the second trial, DNA-repair deficient mutant cells are used. When the test compound is mutagenic or radiomimetic, the DNA-repair deficient mutant cells are killed. The results were that a larger growth inhibition zone is observed as compared with the wild strain (*39,40*). Table 9 lists the bacteria strains used. In the first experiment each two strains of *Escherichia coli* and *Salmonella typhimurium* were used, and in the second trial several strains of *E. coli* (*41*), *Bacillus subtilis* (*42*) and *Salmonella* (*43*) were used, each including both wild strain and DNA-repair deficient mutant.

**Table 9. Bacteria strains used for mutagenicity test of synthetic pyrethroids.**

| | |
|---|---|
| 1. Reversion of amino acid requirement | |
| *Escherchia coli*; w3623 *trp*A$^-$, w3102 *trp*E$^-$ | |
| *Salmonella typhimurium*; TA1535 *his*G$^-$, TA1538 *his*D$^-$ | |
| 2. Inhibition zone of DNA-repair deficient mutant | |
| *Escherichia coli*; | w3623 (wild) |
| | w3623 *pol*A$^-$, w3623 *uvr*A$^-$, w3623 *rec*A$^-$ |
| *Bacillus subtilis*; | H17 (wild) |
| | M45 *rec*A$^-$ |
| *Salmonella typhimurium*; | TA1978 (wild) |
| | TA1538 *uvr*B$^-$ |

**Table 10. Reversion of amino acid requirement of bacteria by pyrethroids.**

| Compound (10 mg/disk) | Colonies of revertants, number/plate | | | |
|---|---|---|---|---|
| | *E. coli* | | *S. typhimurium* | |
| | w3623 | w3102 | TA1535 | TA1538 |
| Control (DSMO, 10 $\mu$l) | 5 | 29 | 14 | 25 |
| Nitrosoguanidine (0.1 mg/plate) | 173 | 1275 | 3000 | 153 |
| 4-NQO (0.01 mg/plate) | 93 | 218 | 231 | 382 |
| Pyrethrin | 5 | 21 | 15 | 27 |
| Allethrin | | | | |
| Racemic | 5 | 15 | 12 | 22 |
| (+)-*trans* | 7 | 37 | 16 | 31 |
| (+)-*cis* | 7 | 40 | 18 | 27 |
| (+)-allethronyl (+)-*trans* | 7 | 27 | 19 | 29 |
| Fenothrin | | | | |
| Racemic | 9 | 25 | 13 | 26 |
| (+)-*trans* | 11 | 35 | 15 | 23 |
| (+)-*cis* | 2 | 32 | 16 | 21 |
| Furamethrin, racemic | 4 | 27 | 23 | 19 |
| Permethrin | | | | |
| Racemic | 9 | 38 | 17 | 29 |
| (+)-*trans* | 7 | 40 | 12 | 32 |
| (+)-*cis.* | 10 | 27 | 17 | 35 |
| (−)-*trans* | 6 | 25 | 15 | 27 |
| (−)-*cis* | 6 | 25 | 19 | 29 |
| Resmethrin | | | | |
| Racemic | 5 | 27 | 11 | 17 |
| (+)-*trans* | 4 | 28 | 12 | 11 |
| (+)-*cis* | 5 | 30 | 24 | 19 |
| (−)-*trans* | 7 | 19 | 16 | 12 |
| (−)-*cis* | 6 | 17 | 16 | 17 |
| Tetramethrin, racemic | 2 | 31 | 9 | 18 |

Table 10 shows the results of the first trial. They indicate clearly that none of the pyrethroids dissolved in dimethyl sulfoxide at a dose of 10 mg/plate are mutagenic under the experimental conditions. On the other hand, nitrosoguanidine at 0.1 mg/plate or 0.01 mg of 4-nitroquinoline *N*-oxide produces very many colonies of the revertant.

One example of the second trail is reproduced in Figure 2. Again, 0.1 mg of nitrosoguanidine forms a significantly larger growth inhibition zone of DNA polymerase A$^-$ strain than the wild strain, while in the case of allethrin at 10 mg or DMSO no halo is observed around the paper disk. The pyrethroids listed in Table 10 were tested, and none of them are mutagenic or radiomimetic.

In the host-mediated assay (*44*), up to one-half the $LD_{50}$ value of each pyrethroid was administered orally to mice. At 3 hr post-treatment, *Salmonella typhimurium* G46 cells were harvested from the abdominal cavity of the animals, and the reversion frequency was determined by inoculating them both in histidineless and histidine-rich media. As the results in Table 11 indicate, none of the tested pyrethroids increase reversion frequency of the bacteria, while streptozotocin remarkably increases the reversion frequency.

Thus, based on the existing toxicological informations, it can be concluded that the toxicity of these synthetic pyrethroids is fairly low for mammals, and that the present recommended use patterns might afford sufficient safety margin for human populations.

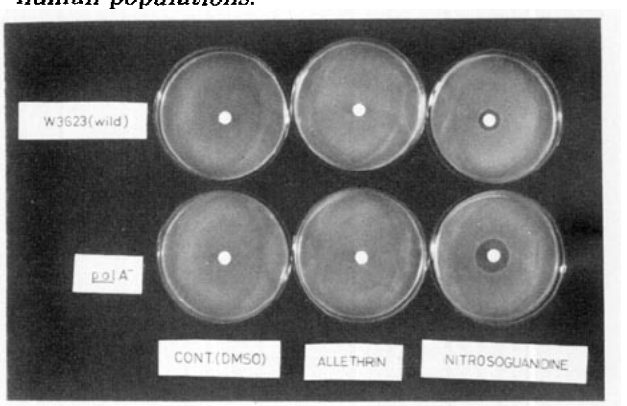



FIGURE 2. Growth inhibition zone of *E. coli* DNA polymerase A-strain and the corresponding wild strain by DMSO (10 $\mu$l), allethrin (10 mg) and nitrosoguanidine (0.1 mg).

Table 11. **Host-mediated assay of pyrethroids in mice on *S. typhimurium* G46.**

| Compound | Dose, mg/kg, PO | Reversion frequency |
|---|---|---|
| Control (corn oil) | 10 ml | $4.1 \times 10^{-7}$ |
| Streptozotocin | 20 | $1.4 \times 10^{-4}$ |
| Pyrethrin | 185 | $2.2 \times 10^{-7}$ |
| Allethrin | 84 | $3.6 \times 10^{-7}$ |
| (+)-*trans* | 165 | $7.7 \times 10^{-7}$ |
| | 66 | $3.3 \times 10^{-7}$ |
| (+)-*cis* | 105 | $5.7 \times 10^{-7}$ |
| | 42 | $9.9 \times 10^{-7}$ |
| (+)-allethronyl | 143 | $8.2 \times 10^{-7}$ |
| (+)-*trans* | 57 | $2.6 \times 10^{-7}$ |
| Permethrin | | |
| (+)-*trans* | 3000 | $5.7 \times 10^{-8}$ |
| | 600 | $1.5 \times 10^{-8}$ |
| (+)-*cis* | 54 | $1.0 \times 10^{-7}$ |
| | 21 | $2.9 \times 10^{-7}$ |
| Resmethrin | | |
| (+)-*trans* | 250 | $1.2 \times 10^{-7}$ |
| | 100 | $8.5 \times 10^{-7}$ |
| (+)-*cis* | 90 | $1.4 \times 10^{-6}$ |
| | 36 | $4.3 \times 10^{-7}$ |

# Toxicity to Fish and Wildlife

It is natural that whenever a compound is to be applied in the outdoor environment, very careful consideration should be required on the possible adverse effects of the compound on fish and wildlife. It seems that there is scanty information for pyrethroids so far, except that the natural pyrethrin mixture has long been known to be toxic to fish and arthropods (*45*) and that, on the contrary, it is relatively harmless to birds (*45,46*). Synthetic pyrethroids are likely to resemble pyrethrin in these respects. Lovebirds are not killed by oral intubation of 2000 mg/kg of racemic allethrin, tetramethrin, (+)-*trans*- and (+)-*cis*-mixture of furamethrin, resmethrin or fenothrin. Only (+)-allethronyl (+)-*trans*-allethrin has $LD_{50}$ of 1600 (male) or 840 (female) mg/kg.

Table 12 reproduces the preliminary data on the toxicity of pyrethroids in fish and arthropods. These compounds are very toxic to killifish, more toxic than organophosphorus compounds and carbamate insecticides. Among the compounds tested,

Table 12. **Toxicity of synthetic pyrethroids in killifish, *Oryzias latipes* and in *Daphonia pulex*.**

| | Toxicity | | | |
|---|---|---|---|---|
| | Killifish (48 hr) | | Daphonia (3 hr) | |
| Compound | TLM, ppm | No-effect level, ppm | TLM, ppm | No-effect level, ppm |
| Pyrethrin | 0.072 | 0.01 | >50 | 0.01 |
| Allethrin | | | | |
| Racemic | 0.087 | 0.005 | >50 | 0.01 |
| (+)-*trans* | 0.050 | 0.005 | 25–50 | 0.01 |
| (+)-*cis* | 0.042 | 0.005 | >50 | 0.01 |
| (+)-allethronyl (+)-*trans* | 0.032 | 0.005 | 5–10 | 0.005 |
| Fenothrin | | | | |
| Racemic | 0.20 | 0.01 | >50 | 0.001 |
| (+)-*trans* | 0.12 | 0.01 | 25–50 | 0.001 |
| (+)-*cis* | 0.17 | 0.001 | >50 | 0.001 |
| (−)-*trans* | >10 | 5 | >50 | 0.05 |
| (−)-*cis* | >10 | 5 | >50 | 0.05 |
| Furamethrin, racemic | 0.18 | 0.01 | >50 | 0.001 |
| Permethrin | | | | |
| Racemic | 0.041 | 0.001 | >50 | 0.001 |
| (+)-*trans* | 0.017 | 0.001 | >50 | 0.001 |
| (+)-*cis* | 0.013 | 0.001 | >50 | 0.001 |
| (−)-*trans* | >10 | 5 | >50 | 0.05 |
| (−)-*cis* | >10 | 5 | >50 | 0.05 |
| Resmethrin | | | | |
| Racemic | 0.30 | 0.001 | >50 | 0.001 |
| (+)-*trans* | 0.016 | 0.001 | 25–50 | 0.0001 |
| (+)-*cis* | 0.008 | 0.001 | 25–50 | 0.0001 |
| (−)-*trans* | >10 | 5 | >50 | 0.05 |
| (−)-*cis* | 3.5 | 0.5 | >50 | 0.05 |
| Tetramethrin | | | | |
| Racemic | 0.20 | 0.5 | >50 | 0.05 |
| (+)-*trans* | 0.20 | 0.05 | >50 | 0.01 |
| (+)-*cis* | 0.15 | 0.05 | >50 | 0.01 |

resmethrin and permethrin are more harmful and (+)-*cis*-resmethrin is extremely toxic. The (−)-isomers are less toxic than the (+)-isomers.

To *Daphnia*, these pyrethroids are much less poisonous, but the no-effect level is again quite low in some compounds. A kind of freshwater shrimp, *Palaemon paucidens* De Haan, seems very susceptible, like killifish, to (+)-*trans*- or (+)-*cis*-resmethrin and (+)-*trans*- or (+)-*cis*-permethrin; TLM in 48 hr for these four compounds being approximately 0.0005 ppm.

Elucidation of mammalian metabolism as well as mode of action of synthetic pyrethroids are fundamental for safety assessment on humans. Fairly extensive metabolic studies have been carried out. However, it is highly desirable and may be necessary to characterize further as many metabolites as possible, and also to extend these studies to other mammalian species, since almost all of the foregoing studies have been limited to albino rats; furthermore, an appreciable numbers of excreted metabolites has not yet been characterized.

There are sufficient mammalian toxicology data on the pyrethroids to support the present use patterns. But, if a use pattern of pyrethroid is to be extended to agricultural pest control, resulting in definite amounts of residues in crops, then supplementary toxicology data such as carcinogenicity and multigeneration reproduction would be required to assess long-term toxicity to humans.

On the other hand, few data are available on the impact of these compounds on the environment. Now that these pyrethroids are inherently very active to some kinds of fish and arthropods as shown above, much more extensive investigations should be forthcoming from both chemical and biological aspects to minimize and hopefully to negate such undesirable side effects on the environment, especially in the use of agricultural pest control.

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